tion (which can reduce linked variation through a 'selective sweep').

Moreover most of the fixed differences occur at positions that alter the amino acid sequence (and hence, potentially, the function) of the protein. In fact, the ratio of non-synonymous (amino-acid altering) to synonymous substitutions in the OR genes is quite high—about 0.74. Although a threshold value of this ratio is often taken to be 1 (ref. 7), the observed value is roughly 3 times larger than the average value calculated from a variety of genes. One of the highest non-synonymous to synonymous (Ka/Ks) ratios observed in humans is at the HLA loci, where positive selection is known to have a major role. And yet, even at these loci, where selective pressures have been quite strong, Ka/Ks exceeds 1 only within the 57 nucleotides of the antigen recognition site; across the entire HLA locus, the Ka/Ks is much lower—roughly equal to 0.40 (ref. 8). Were the odorant-binding portion of the OR genes identified and variability sufficiently high, it might be possible to identify a region of the gene where adaptive evolution was particularly pronounced, with a Ka/Ks greater than 1.

In addition to providing some of the first evidence for adaptive evolution in humans that is not related to infectious disease, the results of Gilad *et al.* provide insights into the evolution of multigene families, and give rise to an interesting new hypothesis. Like the HLA genes, OR genes are members of a multigene family and carry out a similar function to one another. Diversity—provided by the many genes comprising the family—is needed to sense a broad array of chemical odorants. Allelic diversity at most of these loci is also high, particularly at the amino-acid level, and the question is whether this will prove to be a general rule. Does the fact that selection keeps the many members of a multigene family intact also imply that the same selective forces will generate and maintain considerable allelic diversity at each of the loci?

This question highlights a paradox in the evolution of olfactory receptors. If it is true that humans have a reduced sense of smell, and that relaxed selective pressures have allowed many OR genes to deteriorate into pseudogenes, why has positive selection (even if fairly weak) maintained allelic diversity at the intact loci? These days, it is difficult to imagine a circumstance where our own survival has hinged on the ability to sense a particular odorant, but evidence indicating that our choice of mates may be influenced by the sense of smell continues, surprisingly, to accumulate. Studies of rodents and even humans have shown that mate choice is non-random with respect to MHC (HLA) genotype[9,10] (with a preference for mates of a different genotype), and that MHC type influences body odour in a detectable way[11,12]. The obvious question is whether HLA and OR genes may be co-evolving, with variation at one system interacting with variation at the other. Directly detecting such a highly speculative association among these loci is not likely to be possible, when the number of olfactory receptor genes and their alleles is so overwhelmingly large. On the other hand, we shall certainly gain insights into the evolution of OR genes by comparing these patterns observed in humans with species whose selective constraints on OR genes may differ. □

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A debut for mito-mouse

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The inability to construct animal models of human diseases caused by mutated mitochondrial DNA (mtDNA) has been a major obstacle to investigating pathogenetic mechanisms associated with specific mtDNA mutations. Mice carrying a large-scale deletion in mtDNA have now been produced. They display some of the key features of the human disorder, but there are surprising differences.

Energy produced by the mitochondrial respiratory chain is essential for the normal function of most cells in the body. A complete deficiency in respiratory chain function is lethal in early embryonic development[1], but partial deficiencies result in a clinically diverse group of disorders, often referred to as encephalomyopathies[2]. Gene knockout technology has been used to develop mouse models of respiratory chain dysfunction[3,4], but it has not been possible to tackle the fundamental issues in human mitochondrial genetics because no true animal models of mtDNA disease exist. The study by Kimiko Inoue *et al.*[5] on page 176 of this issue therefore comes as a welcome advance. By introducing mtDNAs carrying a large-scale deletion into mouse embryos, they have created the first mouse model that is directly relevant to human mtDNA diseases.

Mitochondrial make-up

The mitochondrial respiratory chain is composed of five multimeric enzyme complexes. Although mtDNA encodes only 13 of the (approximately) 80 structural subunits in these complexes, mutations in this small genome are responsible for the majority of respiratory-chain disorders in adults. Mammalian mtDNA is maternally inherited and is present in hundreds to thousands of copies in most cells. Most afflicted patients have a mixture of mutant and wild-type genomes (mtDNA heteroplasmy) that can vary among tissues and change over time as mitochondria replicate and cells age.

More than 50 different mutations in mtDNA have now been associated with human disease[6,7]. As all mutations ultimately result in decreased energy produc-

tion, one might predict them to produce similar clinical phenotypes. In fact, different clinical phenotypes are associated with specific mtDNA mutations. Moreover, there are marked differences in the patterns of transmission and segregation of specific pathogenic mtDNA mutations.

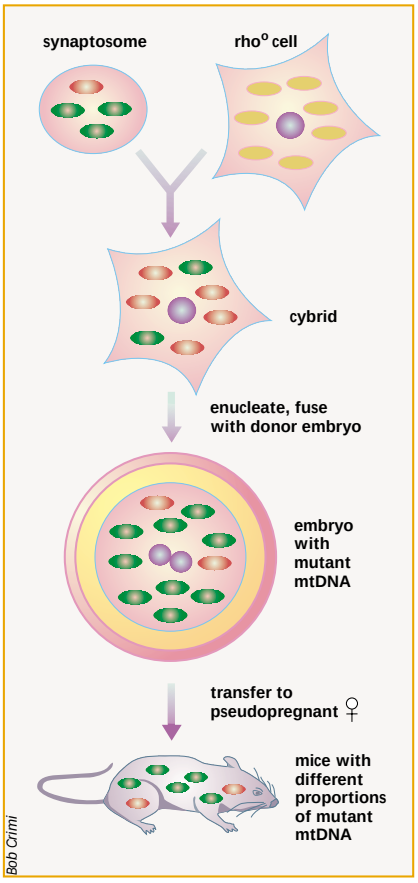
Modelling mtDNA diseases

Why has it been so difficult to produce animal models of human mtDNA diseases? One reason is the inability to stably introduce mutagenized mtDNA into the mitochondria of a mammalian cell. Inoue *et al.*[5] circumvented this problem by isolating naturally occurring somatic mtDNA mutations from ageing mice. Large-scale deletions of mtDNA, similar to those found in human disease, increase with age in both rodents and humans. The deletions usually remove several tRNA genes, producing a mitochondrial translation defect when the relative proportion of mutants in the cell exceeds a certain threshold, about 65% in human cells[8].

To isolate mitochondria containing deleted mtDNAs, Inoue *et al.*[5] prepared synaptosomes (resealed cytoplasmic vesicles from nerve endings) from mouse brain and fused them with rho^o cells, a cell line depleted of mtDNA (see figure). Each fusion event introduces a variable number of mutant and wild-type mtDNAs, which then repopulate the rho^o cell, creating a cytoplasmic hybrid (cybrid) cell line. Cybrid clones were screened by PCR to identify those containing mtDNA deletions, and one clone was identified. The cybrid cells were enucleated, fused to donor embryos, and implanted in pseudopregnant females. Although this procedure produced a low proportion of live births, heteroplasmic founder female animals were identified and, following breeding, germline transmission of the mtDNA deletion was obtained through three generations.

Mouse and human phenotypes

Patients with mtDNA deletions present with syndromes whose severity and age of onset depend on the load and tissue distribution of mutant mtDNA. The most frequent clinical presentation is a combination of complete paralysis of the extraocular muscles (progressive external ophthalmoplegia), droopy eyelids, muscle weakness and exercise intolerance with onset in the third or fourth decade. The hallmark pathology is the segmental accumulation of abnormal mitochondria in skeletal muscle fibres. These 'ragged-red fibres' invariably contain a large proportion of deleted mtDNAs and are negative for markers of respiratory chain function. Kearns-Sayre syndrome (KSS), a more complex neurological syndrome, occurs with higher mutant loads[9]. In addition to the above features, people with KSS also have a block in cardiac conduction, high levels of protein in the cerebro-spinal fluid and pigmentary retinopathy. Diabetes and hearing loss are also common. Pearson syndrome, a much rarer disorder with early onset, is characterized by a severe reduction in all blood cells (pancytopenia) and pancreas dysfunction. These patients have a high proportion of deleted mtDNAs in most tissues, and survivors usually develop features of KSS. All are sporadic diseases.



Manipulating mitochondria. Synaptosomes containing mitochondria with mutated (indicated in red) and wild-type mtDNAs (indicated in green) are fused with rho^o cells, whose mitochondria lack mtDNA. Cybrid cells are enucleated and fused with a donor mouse embryo. The resulting embryos are implanted into pseudopregant females and the offspring are screened for heteroplasmic animals containing both wild-type and deleted mtDNAs.

And the mice? As in human, the heart and skeletal muscle of the mice are mosaics for a marker of respiratory chain function. Ragged-red fibres, however, are not seen and the threshold for expression of a biochemical defect in muscle (about 90% in mutants) is much higher than in human cells. In addition, unlike the human situation, the abnormal skeletal muscle fibres of mutant mice themselves appear to be mosaics. These observations suggest important species differences in the organization and segregation of deleted mtDNAs. Most of the other features of the human phenotype, such as diabetes, hearing loss, exercise intolerance and pancreas dysfunction, were not reported, although the fact that the mice showed signs of anaemia may indicate a problem with blood-cell production. The typical ocular abnormalities, if they exist, would probably be difficult to discern. Unexpectedly, most of the mice died of kidney failure before 200 days of age. Kidney failure has been reported in several patients with mtDNA deletions, but it is not a typical symptom of mitochondrial disease.

There are two other surprising differences between mouse and human. For reasons unknown, germline transmission of mtDNA deletions has rarely been reported in humans[10]. Partial duplications, which can be considered as a dimer of a wild-type and a deleted mtDNA, can be transmitted and may later rearrange to produce deleted and wild-type molecules[11,12]. The authors raise the possibility that the mtDNA deletions in their mice are transmitted as duplications, then rearrange in somatic tissues during development. This hypothesis could be easily tested by investigating the state of mtDNA in the oocytes of transmitting mothers. If true, it suggests extraordinarily tight control of intramolecular recombination of these rearranged mtDNA molecules in germ line versus somatic tissues, as partial duplications were undetectable in most somatic tissues analysed[5].

Similar to previous studies on neutral polymorphic mtDNA sequence variants in mice[13], the mtDNA deletions in mice appear to be transmitted randomly between generations, as demonstrated by the fact that the proportion of mtDNA mutants in mothers was similar to the mean proportion in their offspring. If the mutants are transmitted as mtDNA deletions, this argues against strong selection for respiratory chain function in the female germ line. Remarkably, the variance in the mtDNA genotype frequency is much reduced in the offspring from mothers with very high proportions of mtDNA deletions, an observation that remains unexplained.

A second striking difference lies in the pattern of segregation of deleted mtDNAs. Large differences in the proportion of mutant mtDNAs among tissues are common in human diseases and contribute to differential tissue involvement in the disease phenotype. For instance, mtDNA deletions, which are abundant in muscle of patients with progressive external ophthalmoplegia, are not usually detectable in blood. Individual mice showed similar

proportions of deleted mtDNAs in all tissues, suggesting that the proportion of mutants inherited at birth is more important that tissue-specific segregation during life in the mouse.

The first mouse model of mtDNA disease has raised many interesting questions about the genetics of pathogenic mtDNA mutations. Whether the differences between mouse and human reflect differences in the organization and expression of mtDNA in different species, or particular differences related to mtDNA deletions, will be addressed through further investigation of the current model and the generation of models with mtDNA point mutations. In theory, the latter could be produced using a strategy similar to that employed here, but isolating the appropriate mutations at levels sufficient to create heteroplasmic embryos will be difficult. The holy grail of mammalian mitochondrial genetics, the transformation of mtDNA, remains an important goal. □

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The genomics gamble

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Pair-wise genome comparisons offer new sources of information about the patterns and processes that influence genomic designs. Replication-dependent rearrangements, as indicated by the symmetric gene organization pattern in the genomes of *Chlamydia pneumoniae* and *Chlamydia trachomatis*, may provide a missing link in the reconstruction of historical events from modern genomes.

The neutral theory of molecular evolution[1] describes how God's letters change as a function of time. But to explain how the papers in her office are organized, we have to understand mutational mechanisms other than those that affect single-base substitutions. There are many different kinds of mutations that influence the architecture of a genome, such as duplications, deletions, inversions, translocations and changes that occur as a consequence of horizontal gene transfer (Fig. 1). But our understanding of the rates and mechanisms whereby such architectural modifications occur is still in its infancy, due to a deficiency of sequenced genomes that are sufficiently similar to permit alignment, and yet sufficiently divergent to permit a quantification of their differences. Now, however, we have a possible explanation for how some of these changes may have occurred: on page 195, Elizabeth Tillier and Richard Collins suggest that translocations occur primarily at the replication forks[2].

As such, the hypothesis is notable because it provides a mechanistic link between the two highly similar genomes—those of *Chlamydia trachomatis* and *Chlamydia pneumoniae*—and more distant genomes whose sequences have been obtained. The astonishing feature of the comparative analysis of the *Chlamydia* genomes concerns the location of blocks with similar gene order structures. Tillier and Collins have identified blocks of conserved gene order structures whose position is 'mirror-imaged' across the replication fork (Fig. 2), as also noted by Read and colleagues[3]. This may be a general phenomenon, as such symmetries have also been noted in two strains of *Helicobacter pylori* as well as in the genomes of *Mycobacterium leprae* and *Mycobacterium tuberculosis*[2]. What are the larger implications of the mirror-imaged organization of these genomes? Does it reflect a symmetrical distribution of repeats across the replication axes? Probably not, because the conserved blocks are not flanked by repeats. Does it reflect the timing and close proximity of the replication forks, as suggested by Tillier and Collins? The hypothesis that single-stranded DNA and the double-stranded breaks that occur at the replication fork are especially vulnerable to recombination events makes biological sense. The authors note that the translocation sites are often flanked by inversions or losses and gains of single genes[2], as would be expected were the recombination events mediated by illegitimate recombination or transposons.

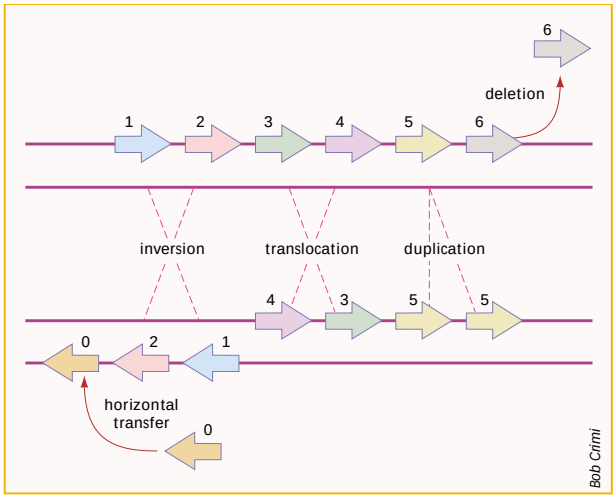

Fig. 1 Schematic illustration of how genomic rearrangements, such as inversions, translocations, duplications, deletions and horizontal gene transfer events, affect the architecture of a genome. Arrows represent a gene or a segment of genes.

Experimental studies are required to nail down details of the link between DNA replication and relocation. It should be stressed, however, that not all inversions and translocations have followed in the footsteps of the replication bubble. Of course, with only two genomes, it is impossible to reconstruct the exact order of events, and several equally possible rearrangement scenarios should be carefully examined when data from additional members of the family become available. Moreover, genomic sisters in other families may provide other mechanistic clues, as rearrangement mechanisms could differ for different